Synthesis of the Selective 5-Hydroxytryptamine 4 (5-HT_4) Receptor Agonist (+)-(*S*)-2-Chloro-5-methoxy-4-[5-(2-piperidylmethyl)-1,2,4-oxadiazol-3-yl]aniline

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In a search for novel 5-hydroxytryptamine 4 (5-HT_4) agonists focusing on the linker group of benzamide derivatives, 2-chloro-5-methoxy-4-[5-(2-piperidylmethyl)-1,2,4-oxadiazol-3-yl]aniline (2) was prepared and its optical isomers were separated. The *S* isomer 2(*S*) showed high affinity for the human 5-HT_4 receptor without affinity for the human 5-HT_3 receptor, and potent 5-HT_4 agonistic activity in longitudinal muscle myenteric plexus (LMMP) preparations of guinea pig ileum. The *R* isomer 2(*R*) showed opposite selectivity. As a result of other receptor binding studies, 2(*S*) (YM-53389) was shown to be a highly selective 5-HT_4 agonist.

Key words 5-hydroxytryptamine 4 agonist; 1,2,4-oxadiazole; *S* isomer

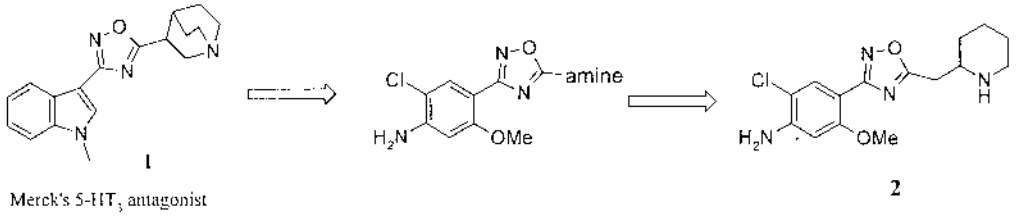
Serotonin (5-hydroxytryptamine, 5-HT) is widely distributed in the central nervous, gastrointestinal, and cardiovascular systems as a neurotransmitter, neuromodulator and hormone.[1—6] The 5-hydroxytryptamine 4 (5-HT_4) receptor is distributed from the esophagus to the colon in the gastrointestinal system, and is especially closely related with regulation of gastrointestinal motility.[7] For these reasons, 5-HT_4 agonists are expected to be effective for treatment of gastrointestinal dysfunctions such as diarrhea, constipation, gastroparesis, ileus, reflux esophagitis, and pseudo-obstructions.

Most of the reported 5-HT_4 agonists, except for 5-HT derivatives,[8,9] have a benzamide (or other arylcarboxamide) skeleton,[7] but many possess poor selectivity for the 5-HT_4 receptor due to 5-HT_3 antagonistic activity.

In order to find new 5-HT_4 agonists, we focused on the linker group of the benzamide and synthesized 3-(4-amino-5-chloro-2-methoxyphenyl)-1,2,4-oxadiazole derivatives, derived from the Merck 5-HT_3 antagonist **1** possessing an oxadiazole ring (Chart 1).[10] Consequently, it was found that a 1,2,4-oxadiazole ring could function as a linker group instead of an amide bond for 5-HT_4 agonists as well as for 5-HT_3 antagonists. Among such derivatives, cyclic amine derivatives (quinuclidines, hexahydropyrrolizines, piperidines, pyrrolidines) having a basic nitrogen atom at the γ-position from oxadiazole showed potent 5-HT_4 agonistic activity in the longitudinal muscle myenteric plexus (LMMP) preparation of guinea pig ileum, and especially 3-piperidylmethyl derivative (**2**) showed a unique profile. In this paper, we report the synthesis, potency, and receptor selectivity of (*S*)-2-chloro-5-methoxy-4-[5-(2-piperidylmethyl)-1,2,4-oxadiazol-3-yl]aniline [**2**(*S*)] as a 5-HT_4 agonist.

The oxadiazole derivatives were synthesized as follows (Chart 2). Benzamide (**4**), prepared from benzotriazolyl ester (**3**)[11] and aqueous ammonia in *N*,*N*-dimethylformamide (DMF), was converted into nitrile (**5**) by dehydration with phosphorus oxychloride. *N*-Hydroxybenzamidine (**6**), obtained from **5** and hydroxylamine in refluxing ethanol, was cyclized with aminoesters in the presence of sodium hydride in DMF to afford 3-(4-amino-5-chloro-2-methoxyphenyl)-1,2,4-oxadiazole derivatives. Racemic compound **2**(*RS*) was obtained from methyl 2-piperidineacetate, and its optical isomers [**2**(*S*), **2**(*R*)] were separated by optical resolution of the correspond ditoluoyltartaric acid salts recrystallized from DMF–H_2O. The absolute configuration of **2**(*S*) grown from AcOEt–EtOH was determined on the basis of X-ray structure analysis.

Receptor selectivities of **2**(*S*), **2**(*R*), and cisapride[12] were evaluated by the binding affinities using guinea pig 5-HT_4 receptor, rat 5-HT_3 receptor, and cloned human 5-HT_3[13] and 5-HT_4 receptors,[14] and 5-HT_4 agonistic activities were measured by LMMP preparation (Table 1).[15—17] Whereas Schiavi *et al*. previously reported the receptor selectivity of 5-HT_4 agonists,[18] only cisapride and SC-53116 showed higher affinities for the 5-HT_4 receptor (pig caudate nucleus) than the 5-HT_3 receptor (NG 108-15 hybrid cells), and the 5-HT_3/5-HT_4 K_i ratios were 4.2 and 2.5, respectively.[18] In our study, the *S* isomer **2**(*S*) (YM-53389) showed 9 times higher affinity for the 5-HT_4 receptor than the 5-HT_3 receptor in guinea pig and rat, whereas **2**(*R*) showed the opposite selectivity. YM-53389 retained high affinity for the human 5-HT_4 receptor but had no affinity for the human 5-HT_3 receptor. The receptor selectivity of YM-53389 was vastly superior to that of cisapride in our binding study, and this compound showed a potent stimulatory effect for 5-HT_4 agonistic activ-



1 Merck's 5-HT_3 antagonist **2**

Chart 1

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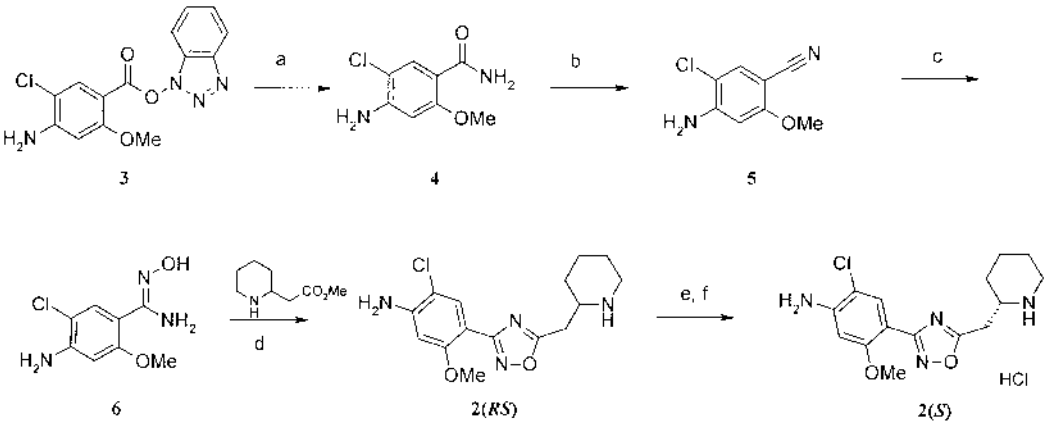

Chart 2

Table 1. Binding Affinities for 5-HT_4 and 5-HT_3 Receptors, and 5-HT_4 Receptor Agonism

Compound	5-HT_4 receptors K_i (nM)[a]		5-HT_3 receptors K_i (nM)[a]		5-HT_4 agonism EC_{50} (μM)[b]
	Guinea pig striatum	Human	Rat cortex	Human	
2(*S*)(YM-53389)	82	54	735	>10000	0.50
2(*R*)	303	NT	172	NT	NT
Cisapride	96	42	61	684	0.15
	(33)[c]		(139)[d]		
SC-53116	(21)[c]	—	(51)[d]	—	—

a) [^{3}H]-GR113808 and [^{3}H]-ramosetron were used as 5-HT_4 and 5-HT_3 receptor ligands, respectively. *b*) 5-HT_4 agonistic activities were measured using LMMP of guinea pig ileum. The results were expressed as EC_{50} values, which were calculated by subtracting the twitch responses obtained under the 5-methoxytriptamine (5-MOT; 5-HT_4 agonist)-desensitized condition from these obtained with the tested compound alone. *c*) Pig caudate nucleus, [^{3}H]-GR113808.[18] *d*) NG 108-15 hybrid cells, [^{3}H]-DAU6215.[18]

Table 2. Binding Profiles of **2**(*RS*) for Other Receptors[a]

Binding site	Ligand	K_i (nM)	
		2(*RS*)	Cisapride
Adrenaline α_1	[^{3}H]-Prazosin	>10000	79
Adrenaline α_2	[^{3}H]-Rauwolscine	>10000	2700
Muscarine M_1	[^{3}H]-Pirenzepine	>10000	5400
5-HT_{1A}	[^{3}H]-8-OH-DPAT[b]	>10000	1600
5-HT_2	[^{3}H]-Ketanserin	>10000	8.7
Dopamine D_2	[^{3}H]-YM-09151-2	>10000	50
Sigma	[^{3}H]-DTG[b]	540	110

a) Racemic **2**(*RS*) and cisapride were inactive at the following receptors (K_i, >10000 nM): Adenosine A_1, A_2, adrenaline β_1, β_2, angiotensin II, bombesin, bradykinin B_2, CCK_A, CCK_B, dopamine D_1, histamine H_1, H_3, muscarine M_2, M_3, M_4, neurokinin NK_1, neuropeptide Y_2, opiate δ, κ, μ, and VIP. *b*) 8-HO-DPAT, 8-hydroxy-2-dipropylaminotetralin; DTG, 1,3-di(2-tolyl)guanidine.

ity in the LMMP preparation.

Next, the binding affinities for other receptors were measured to reveal the pharmacological profile of **2**(*RS*) and cisapride (Table 2). All receptor binding studies were performed by standard procedures using standard assay conditions. As a result, **2**(*RS*) showed only weak affinity for the sigma receptor, but no affinity to other 5-HT receptor subtypes and the receptors of other transmitters. These results indicate that YM-53389 is a highly selective 5-HT_4 receptor ligand. This compound is undergoing further evaluation due to the structural novelty, in comparison to benzamide YM-47813, described in the preceding report and to its good pharmacological properties.

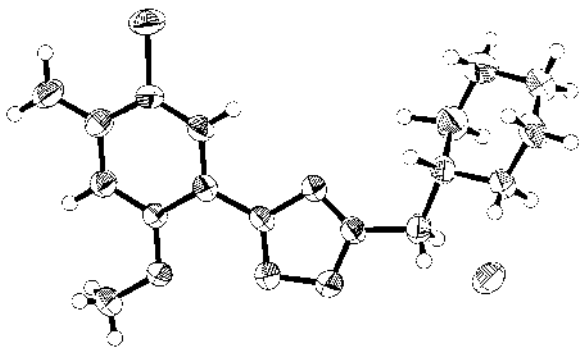

Fig. 1. X-Ray Structure of YM-53389

Experimental

All melting points were determined on a Yanaco MP-500D melting point apparatus and are uncorrected. ^{1}H-NMR spectra were measured with a JEOL FX90Q, a FX100, a FX270 or FX400 spectrometer; chemical shifts are reported in δ units using tetramethylsilane as internal standard and the following abbreviations are used: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, dd=double doublet, dt=double triplet, br=broad. Mass spectra were recorded with a Hitachi M-80 electron impact (EI), JEOL JMS-DX300 (FAB) spectrometer or Hewlett Packard 5970 MSD (GC) spectrometer. Elemental analyses were performed with a Yanaco MT-5. All organic extracts were dried over anhydrous magnesium sulfate and concentrated with a rotary evaporator under reduced pressure.

4-Amino-5-chloro-2-methoxybenzamide (4) Twenty ml of 29% aqueous ammonia was added to a solution of benzotriazolyl 4-amino-5-chloro-2-methoxybenzoate (**3**) (6.36 g, 20 mmol) in DMF (300 ml) at room temperature and the resulting mixture was stirred for 30 min. The reaction mixture was concentrated and the residue was diluted with AcOEt and aqueous K_2CO_3 solution. The precipitates were collected by filtration and washed

with H_2O, EtOH and AcOEt to afford **4** as a beige solid (3.65 g, 91%). ^{1}H-NMR (DMSO-d_6) δ: 3.82 (3H, s), 5.91 (2H, br s), 6.47 (1H, s), 7.22 (1H, br s), 7.37 (1H, br s), 7.70 (1H, s). EI-MS *m/z*: 200, 202 (M^+).

4-Amino-5-chloro-2-methoxybenzonitrile (5) A suspension of **4** (11.00 g, 55 mmol) in phosphorus oxychloride (50 ml) was stirred at 50 °C for 30 min. The reaction mixture was concentrated and the residue was diluted with H_2O and made alkaline by K_2CO_3. This mixture was extracted with AcOEt. The extract was washed with brine, dried, and concentrated. The residue was crystallized from hexane–AcOEt and **5** was obtained as a beige solid (7.46 g, 75%). ^{1}H-NMR (DMSO-d_6) δ: 3.81 (3H, s), 6.39 (2H, br s), 6.50 (1H, s), 7.53 (1H, s). EI-MS *m/z*: 182, 184 (M^+).

4-Amino-5-chloro-*N*-hydroxy-2-methoxybenzamidine (6) A mixture of **5** (10.32 g, 54 mmol), hydroxylamine hydrochloride (7.93 g, 114 mmol), and anhydrous K_2CO_3 (15.73 g) in EtOH was refluxed for 20 h. The reaction mixture was diluted with H_2O and the precipitate was collected by filtration and washed with H_2O and EtOH to afford **6** as a beige solid (10.72 g, 75%). ^{1}H-NMR (DMSO-d_6) δ: 3.72 (3H, s), 5.48 (2H, br s), 6.30 (2H, br s), 6.48 (1H, s), 7.23 (1H, s), 9.23 (1H, br s). EI-MS *m/z*: 215, 217 (M^+).

2-Chloro-5-methoxy-4-[5-(2-piperidylmethyl)-1,2,4-oxadiazol-3-yl]-aniline (2) A solution of **6** (2.15 g, 10 mmol) in DMF (7 ml) was added dropwise into a suspension of methyl 2-(2-piperidyl)acetate (2.04 g, 13 mmol), and 60% sodium hydride (in oil) (0.50 g, 12.5 mmol) in DMF (15 ml) under water-cooling. The reaction mixture was stirred for 1 h at room temperature. The mixture was then quenched with ice and concentrated. The residue was diluted with water and extracted with AcOEt. The extract was washed with brine, dried, and concentrated. The residue was chromatographed on SiO_2 eluting with $CHCl_3$–MeOH (10 : 1) to afford **2** as a white solid (2.32 g, 72%). mp 101—103 °C (AcOEt–hexane). ^{1}H-NMR ($CDCl_3$) δ: 1.00—2.00 (6H, m), 2.60—3.20 (5H, m), 3.91 (3H, s), 4.36 (2H, br s), 6.38 (1H, s), 7.96 (1H, s). EI-MS *m/z*: 322, 324 (M^+). *Anal*. Calcd for $C_{15}H_{19}ClN_2O_2$: C, 55.81; H, 5.93; Cl, 10.98; N, 17.36. Found: C, 55.53; H, 5.89; Cl, 11.14; N, 17.34.

(−)-(*R*)-2-Chloro-5-methoxy-4-[5-(2-piperidylmethyl)-1,2,4-oxadiazol-3-yl]aniline Monohydrochloride [2(*R*)] A suspension of racemic **2** (48.0 g, 149 mmol) and L-ditoluoyltartaric acid (57.5 g, 149 mmol) in DMF (750 ml) was warmed at 50 °C and dissolved. After removing the oil bath, H_2O (1000 ml) was added to the solution and the resulting mixture was gradually cooled to room temperature and allowed to stand overnight. Precipitate was collected and recrystallized from DMF–H_2O (1 : 1) (total: 3 recrystallizations). The L-ditoluoyltartaric acid salt of **2**(*R*) was added to a mixture of 1 N HCl (80 ml) and AcOEt (100 ml) and stirred for 2 h. The mixture was extracted with diluted HCl. The water layer was made alkaline with K_2CO_3 and extracted with $CHCl_3$–MeOH (10 : 1). The extract was washed with brine, dried, and concentrated. To the residue in EtOH (100 ml) was added 4 N HCl–AcOEt solution (15 ml) and AcOEt (50 ml), and the mixture allowed to stand overnight at room temperature. The precipitate was collected to afford the HCl salt of **2**(*R*) (16.53 g, 31%), mp 215—217 °C (EtOH–AcOEt). *Anal*. Calcd for $C_{15}H_{19}ClN_4O_2\cdot HCl$: C, 50.15; H, 5.61; Cl, 19.74; N, 15.60. Found: C, 50.02; H, 5.60; Cl, 19.55; N, 15.69. $[\alpha]_D^{20} = -14.8$ (c=1.00, MeOH).

(+)-(*S*)-2-Chloro-5-methoxy-4-[5-(2-piperidylmethyl)-1,2,4-oxadiazol-3-yl]aniline Monohydrochloride [2(*S*)] The filtrate from the first recrystallization was recovered and 22.0 g of crude free base **2**(*S*) (*S* : *R*=88 : 12) was obtained. This crude **2**(*S*) was purified by optical resolution with D-ditoluoyltartaric acid and converted into the HCl salt of **2**(*S*) (18.9 g, 35% from **2**) according to the above procedure, mp 217—218 °C (EtOH–AcOEt). ^{1}H-NMR (DMSO-d_6) δ: 1.45—1.90 (6H, m), 2.93 (1H, dt, J=3, 12 Hz), 3.25—3.40 (3H, m), 3.46 (1H, dd, J=6, 16 Hz), 3.55—3.65 (1H, m), 6.03 (2H, s), 6.59 (1H, s), 7.74 (1H, s), 9.33 (2H, br). FAB-MS *m/z*: 323, 325 (M^++1). *Anal*. Calcd for $C_{15}H_{19}ClN_4O_2\cdot HCl$: C, 50.15; H, 5.61; Cl, 19.74; N, 15.60. Found: C, 49.91; H, 5.58; Cl, 19.95; N, 15.51. $[\alpha]_D^{20} = +14.9$ (c=1.00, MeOH).

X-Ray Crystallography of YM-53389 Crystals of YM-53389 were grown from AcOEt–EtOH as colorless prisms. Diffraction intensities were collected from a crystal of dimensions 0.52×0.38×0.15 mm on a Rigaku AFC7R four-circle diffractometer. Of the total of 2602 unique reflections (complete for $2\theta<120°$), 1263 satisfied the criterion $F>3\sigma(F)$ and only these were used in the solution and refinement of the structure. Crystal data: $C_{15}H_{20}Cl_2N_4O_2$, M.W.=359.25, monoclinic, space group $P2_1/c$, a=15.949 (1) Å, b=7.727(3) Å, c=15.203(2) Å, β=110.631(6)°, V=1753.3(5) Å^3, Z=4, D_c=1.36 g/cm^3, F_{000}=752.00, CuK_α radiation, graphite-monochromated, λ=1.54178 Å.

Structure Solution and Refinement: The structure was solved by a direct method using SAPI91, and the final refinement was done by the full-matrix least-squares method with anisotropic thermal parameters for all non-hydrogen atoms and fixed isotropic thermal parameters for all hydrogen atoms. The final R value was 0.047. The absolute configuration was determined to be *S* using the method described by Flack [χ=0.01(1)].

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